



# Elevated CO<sub>2</sub> compensates for drought effects in lemon saplings via stomatal downregulation, increased soil moisture, and increased wood carbon storage

Indira Paudel<sup>a,c,d</sup>, Moshe Halpern<sup>b,c</sup>, Yael Wagner<sup>a,d</sup>, Eran Raveh<sup>b</sup>, Uri Yermiyahu<sup>b</sup>, Guenter Hoch<sup>e</sup>, Tamir Klein<sup>a,d,\*</sup>

<sup>a</sup> Institute of Soil, Water, and Environmental Sciences, ARO Volcani Center, Beit Dagan, Israel

<sup>b</sup> ARO Gilat Research Center, M.P. Negev, Israel

<sup>c</sup> The Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture, Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel

<sup>d</sup> Department of Plant & Environmental Sciences, Weizmann Institute of Science, Rehovot 76100, Israel

<sup>e</sup> Department of Environmental Sciences – Botany, University of Basel, Basel 4056, Switzerland

## ARTICLE INFO

### Keywords:

CO<sub>2</sub>  
Storage carbohydrates  
Drought resistance  
Stomatal adjustment

## ABSTRACT

Tree growth enhancement under elevated [CO<sub>2</sub>] is much smaller than originally anticipated; yet carbon overabundance can lead to increased wood carbon storage and to stomatal downregulation and hence reduced water-use. Notably, all three outcomes increase tree drought resistance. Here we studied growth, water relations, and nonstructural carbohydrates of 60 lemon saplings growing in CO<sub>2</sub>-controlled rooms at the same greenhouse, under 400, 650, and 850 ppm [CO<sub>2</sub>]. At each [CO<sub>2</sub>] level, 10 saplings were exposed to 1-month dry-down after 2 months of standard irrigation, followed by re-watering for another month. The other 10 saplings served as controls. Under drought, tree growth was maintained at elevated, but not ambient, CO<sub>2</sub>, linked with mild vs. severe tree water stress (leaf water potential of −3.5 at elevated and −5.5 MPa at ambient [CO<sub>2</sub>]). Stomatal downregulation with increasing [CO<sub>2</sub>] meant that leaf transpiration and diurnal plant water-use were 13–46% lower at elevated vs. ambient [CO<sub>2</sub>] but photosynthesis was still 15–25% higher. CO<sub>2</sub>-induced increases in root and shoot starch were transient but significant. Our results suggest that when predicting tree growth in a warmer and drier future, concomitant atmospheric CO<sub>2</sub> concentration must be considered. In young lemon trees, elevated CO<sub>2</sub> partially compensated for drought effects on tree growth and water status, and might delay some of the effects of the anthropogenic climate change.

## 1. Introduction

Among global environmental changes, the rapid, on-going increase in the atmospheric concentration of CO<sub>2</sub> is one of the highest certainties for the 21st century (Scott et al., 2013). In plants growing under current conditions, photosynthesis is under-saturated at 400 ppm CO<sub>2</sub>, and hence there is an expectation for enhanced carbon (C) sequestration by the future terrestrial biosphere (Jones and Donnelly, 2004; Sholtis et al., 2004). However limitations on the availability of other resources (e.g. light, water, nutrients), and the competition over these resources among individual plants mean that plant C uptake and productivity are not altered at elevated CO<sub>2</sub> in many ecosystems (Körner, 2003; Körner et al., 2005; Klein et al., 2016a). Nevertheless, higher atmospheric CO<sub>2</sub> concentrations allow plants to reduce water losses per unit of carbon

gain, in part by reducing stomatal conductance when the gradient of CO<sub>2</sub> between the atmosphere and the leaf interior increases (Swann et al., 2016). Thus, plant water and C economies are intertwined in multiple ways, from leaf gas exchange (Klein et al., 2016b) to xylem-phloem interactions (Savage et al., 2016). Increased CO<sub>2</sub> can reduce leaf gas exchange, thereby acting as a water-saver allowing plants to increase their water-use efficiency (e.g. Liang and Maruyama, 1995; Maseyk et al., 2008; Keenan et al., 2013). Alternatively, surplus assimilates are stored as nonstructural carbohydrates (NSC), in turn acting as osmoregulators or as a carbon source, extending tree survival under drought (Obrien et al., 2014). It is therefore hypothesized that increased CO<sub>2</sub> levels should promote drought resistance among plants and ecosystems. Preliminary evidence for this hypothesis comes from several studies (e.g. Tschaplinski et al., 1993; Widodo et al., 2003;

**Abbreviations:** NSC, nonstructural carbohydrates; SWC, soil water content; LWP, leaf water potential; g<sub>s</sub>, stomatal conductance; Tr, transpiration; ET, evapotranspiration; Pn, photosynthesis

\* Corresponding author at: Department of Plant & Environmental Sciences, Weizmann Institute of Science, 76100 Rehovot, Israel.

E-mail address: [tamir.klein@weizmann.ac.il](mailto:tamir.klein@weizmann.ac.il) (T. Klein).

<https://doi.org/10.1016/j.envexpbot.2018.01.004>

Received 16 October 2017; Received in revised form 9 January 2018; Accepted 10 January 2018

Available online 31 January 2018

0098-8472/ © 2018 Elsevier B.V. All rights reserved.

Robredo et al., 2007; Zinta et al., 2014) but not from others (Beerling et al., 1996; Duan et al., 2014), suggesting that the effect of high CO<sub>2</sub> on drought resistance might be species- and/or ecosystem-specific (Guehl et al., 1994; Beerling et al., 1996; Franks et al., 2013).

To understand why tree drought resistance can be promoted by elevated CO<sub>2</sub> levels in some tree species but not in others, the underlying mechanisms must be investigated and characterized (Roy et al., 2016; Domec et al., 2017). As mentioned above, one of the pathways is increased C storage. To benefit from C reserves during drought, a tree must have: (1) the regulatory and enzymatic tools to enhance starch synthesis, sometimes in favour of active growth; (2) sufficient storage space, distributed across different compartments; (3) an active, proliferated carbon re-mobilization network, across phloem and ray parenchyma tissues, which must maintain a threshold hydration level to ensure functionality; and (5) the regulatory and enzymatic tools to match starch degradation rates with C demands (maintenance respiration of specific organs) (Hartmann and Trumbore, 2016; Klein and Hoch, 2015). A successful strategy can be accomplished once such requirements have been fulfilled at the whole tree level. At the ecosystem scale, successful strategies in dominant tree species can be translated into higher resilience, ensuring increased survival ratios in spite of the drought and warming conditions. While not increasing productivity, the increased safety, even if limited to certain species, is highly important considering the recent, large-scale drought-induced tree mortality cases (Sarris et al., 2007; West et al., 2008; Klein, 2015) and the expected higher frequency and severity of drought in the future.

Agricultural systems such as fruit tree orchards might be more sensitive to elevated [CO<sub>2</sub>] than natural ecosystems, due to the higher availability of light, water, and nutrients (Culotta, 1995). The availability of these resources is ensured through optimal spacing (Friedman, 2016) on the one hand, and supplemental additions (i.e. fertilization, irrigation, and artificial light) on the other hand. Elevated [CO<sub>2</sub>] has been applied in greenhouses and has been shown to increase yields, e.g. of soybean and tomato (Ainsworth et al., 2002; De Gelder et al., 2005; respectively). The near-optimal growth conditions in orchards seem to preclude the option of drought effects on fruit trees. However in practice, many fruit crops have deficit-irrigation or none entirely, even in a highly water-limited climate (Ortuño et al., 2006; Rodríguez-Gamir et al., 2010). For example, olive is grown in Mediterranean climate, usually without irrigation. In lemon, irrigation is sometimes halted for 1–2 months at the peak of the dry season, to induce flowering and a subsequent, second crop (Raveh, 2006). Drought stress in orchards is not limited to the Mediterranean: in temperate climate, where orchards are seldom irrigated, heat waves can develop rapidly during the summer and affect trees. Climate extremes such as the 2003 summer in Europe, which severely affected crop yields (Van der Velde et al., 2012) can have higher impact on trees than changes in the mean temperature and humidity (Reyer et al., 2013). Since both warming and increase in the frequency and severity of climate extremes are increasing, fruit trees of the future will grow under elevated [CO<sub>2</sub>] on the one hand, and experience more drought stress on the other hand.

In order to test the interactions between the effects of elevated [CO<sub>2</sub>] and drought on fruit tree growth and physiology, we grew 60 one-year old lemon saplings at either 400, 650, or 850 ppm [CO<sub>2</sub>] during a four-month period. After two months, half of the saplings in each CO<sub>2</sub> treatment were subjected to a 1-month drought cycle. To provide the context of mature lemon tree responses, our experiment was complemented by additional measurements in the orchard and on potted, 4-year-old trees. Considering the physiological mechanisms discussed above, we hypothesized that the elevated CO<sub>2</sub> would compensate for drought effects on tree growth via reduced transpiration and increased water availability, due to stomatal downregulation, and via increased C reserves.

## 2. Material and methods

To test the interaction between elevated CO<sub>2</sub> and drought effects on lemon tree physiology, a greenhouse experiment was conducted on 1-year-old lemon saplings. In addition to this main experiment, we bring results from related experiments on 4-years-old and 20-years-old lemon trees, which were performed in parallel. These additional data provide the context of mature trees in the orchard.

### 2.1. Experiment setup and plant material

The study was conducted in closed greenhouse compartments at the Gilat Research Station in southern Israel during January–May 2016. The facility consists of three 2 × 5 m, 4 m tall closed-system rooms that can expose plants to different climatic and atmospheric conditions. Computer-controlled temperature and CO<sub>2</sub> supply system (Emproco Ltd., Ashkelon, Israel) was adjusted automatically inside the rooms every minute to achieve a specific [CO<sub>2</sub>] enrichment and a temperature set point (26 °C during daytime and 16 °C at night). The specific treatments consisted of a set of six treatment combinations of drought (control vs. drought) and elevated CO<sub>2</sub> ([400], [650], and [850] ppm). The ambient CO<sub>2</sub> concentration target was 400 μmol mol<sup>-1</sup> and the elevated CO<sub>2</sub> target were 650 and 850 μmol mol<sup>-1</sup>. Meteorological data such as photon flux density in the photosynthetically active radiation wavebands, air and soil temperatures, air humidity, air vapor pressure deficit (D) and soil moisture were recorded at the site both in and outside the rooms throughout the study. Unlike the [CO<sub>2</sub>] and temperature control, other parameters such as relative humidity (RH) and light conditions were affected by ambient conditions and reflect the seasonal changes during the period of the experiment. Therefore, all saplings were equally exposed to the gradual seasonal decrease in RH (daily minimum RH 50–60% in Jan–Mar and 40% in Mar–May) and increase in light (daily maximum solar radiation 300–500 W m<sup>-2</sup> in Jan–Mar and 500–700 W m<sup>-2</sup> in Mar–May).

Sixty 1-year old lemon saplings (*Citrus limon* (L.) Burm. F. var. ‘Villafranca’), grafted on *C. volkameriana* (Ten. and Parq) rootstock were grown in sandy loam soil within 7 L pots. Saplings were 85 ± 5 cm tall with stem diameter of 10 ± 3 cm. Evergreen lemon saplings has major leaf flushing in spring (March) and autumn (September), although it can partially flush throughout the year. As mentioned above, control rooms were inside the greenhouse and conditions were similar among them, and hence each sapling is considered a single replicate. Sandy loam retains nutrients and water, while still allowing excess water to drain away, and hence considered ideal for citrus cultivation, better than heavier soils (Li et al., 2005; Paudel et al., 2016). It is the major soil type in Israel’s citrus plantations, from the coastal plain to the Northern Negev. Pot volume was chosen to avoid a pot binding effect (Poorter et al., 2012). Indeed, pot dimensions did not limit sapling growth, as evidenced from: (1) comparable gas exchange values to those measured in lemon trees in the orchard (Ortuño et al., 2006; Rodríguez-Gamir et al., 2010); (2) no effect of root mass on stomatal conductance (Fig. S1); and (3) no rhizosheets at the pot bottom and sides. Root biomass varied among plants between 17 and 46 mg, indicating different levels of root growth rather than congregation around a maximum value. Furthermore, root biomass was 55–60% of shoot biomass, similar to ratios measured in an experiment on lemon saplings in 27 L pots (Martin et al., 1995).

Saplings were equally divided between three adjacent greenhouse compartments, set to atmospheric CO<sub>2</sub> concentrations of 400, 650, and 850 ppm CO<sub>2</sub>. In each compartment, ten saplings were exposed to 1-month (week 9–12) dry-down after 2 months of standard irrigation (week 1–8), followed by re-watering for another month (week 13–16). The other ten saplings served as controls. To avoid small biases in growth conditions among saplings, pot locations in each compartment were swapped every two weeks. Although young, saplings were producing fruit buds, which have been removed to avoid shifts due to

changes in resource allocation in fruiting trees. The same amount of water was added to each tree by two drippers every other day through an automated irrigation system until the drought initiation. At the beginning of the experiment, the standard irrigation was  $0.5 \text{ L day}^{-1}$ . After 2 weeks it became apparent that the soil was getting saturated, and the irrigation was reduced to  $0.5 \text{ L}$  every other day till end of the experimental period in all treatments. Irrigation was completely stopped during the drought period, and re-watering provided the same amount of water as control.

## 2.2. Soil and water parameters

Soil water content in volumetric basis (SWC, % v/v) was measured using a dielectric constant EC-10 soil moisture sensor (Decagon devices Inc., Pullman, WA, USA). Measurements were performed on all sixty pots at each of four measurement days: twice before the dry-down cycle, once during the dry-down, and once after re-watering. To correct for sensor bias, sensor readings in sandy loam were calibrated against measured SWC in the standard oven drying procedure using an additional soil batch. The correlation between sensor readings and true SWC yielded a linear relationship, yet with a roughly constant 6.5% underestimation by the sensor ( $R^2 = 0.97$ ,  $0 < \text{SWC} < 45\% \text{ v/v}$ ), and the sensor readings were corrected accordingly. Plant water use was measured weekly using the evaporative flux method. Each pot was weighted on an electronic balance 2 h after irrigation and again after 24 h, and diurnal evapotranspiration was subsequently quantified ( $\text{L day}^{-1}$ ).

## 2.3. Leaf gas exchange and water potential

Leaf  $\text{CO}_2$  and  $\text{H}_2\text{O}$  exchange measurements were done in young matured leaves using LI-6400XT photosynthesis systems (Li-Cor, Inc., Lincoln, NE, USA) equipped with a standard  $2 \times 3 \text{ cm}$  leaf cuvette at ambient leaf temperature of  $25^\circ\text{C}$ , 50% relative humidity, light  $1200 \mu\text{mol m}^{-2} \text{ s}^{-1}$ , 10% blue light,  $400 \mu\text{mol CO}_2 \text{ mol}^{-1}$ . Immediately after a measurement at the prevailing  $[\text{CO}_2]$  in the ambient  $[\text{CO}_2]$  chambers (i.e.,  $400 \mu\text{mol mol}^{-1}$ ), an additional measurement at the reverse  $[\text{CO}_2]$  treatment ( $\sim 650$  and  $850 \mu\text{mol mol}^{-1}$ ) was also taken to evaluate the instantaneous photosynthetic enhancement, expressed as the ratio of  $P_n$  of ambient  $[\text{CO}_2]$  grown plants measured at 650 vs 400 or 850 vs 400  $[\text{CO}_2]$ . This ratio reflects the short-term enhancement of  $P_n$  by elevated  $[\text{CO}_2]$ . These measurements were performed biweekly and measurements were performed on fully grown leaves in late morning between 10:00 to 12:00. In addition,  $P_n$  sensitivity to  $[\text{CO}_2]$  was measured on week 5 by exposing trees to cuvette  $[\text{CO}_2]$  of 0, 50, 100, 200, 300, 400, 650 and 850 ppm. Concurrently with gas exchange measurements, leaf water potential (LWP) was measured on 4 randomly selected plants per treatment. Leaves were collected around 12:00 for mid-day measurements. LWP was either measured on the same leaves as used for gas exchange measurements or an adjacent leaf. Following cutting, LWP was measured within 2 min of collection using a Scholander-type pressure chamber (ARIMAD 2, MRC Ltd., Holon, Israel).

## 2.4. Tree growth and biomass distribution

Plant height and stem diameter (5 cm above and below the grafts) were measured weekly. Plant height was measured using measuring tape and stem diameter was measured by micro-gauge micrometer. To minimize changes in vegetative growth due to reproductive growth, flower buds were removed from plants as soon as identified. Organ specific biomass distribution (fine roots of  $< 2.5 \text{ mm}$  diameter; coarse roots; stem and branches; foliage) was measured at the end of the experiment following the harvest of all saplings. Each sapling was cut from the base of the stem, roots were separated from soil after washing, and then divided between fine and coarse roots based on measured size.

All plant material was oven dried at  $60^\circ\text{C}$  for a week and then biomass was measured using an electronic balance.

## 2.5. Plant nutrition status

The effect of  $\text{CO}_2$  and plant mineral nutrition enrichments is often synergistic (e.g. Weiss et al., 2009, 2010) and hence potential differences in plant nutrition status were assessed. Dry leaf tissue from each was ground and stored pending chemical analysis. Total N, P and K concentrations were determined following digestion with sulfuric acid and peroxide (Snell and Snell, 1949). N and P levels were measured using an automated photometric analyzer (Gallery Plus, Thermo Scientific), and K by an atomic absorption spectrophotometer (AAnalyst 800, Perkin Elmer, Waltham, MA). Each element concentration was calculated per leaf dry weight and per total leaf area.

## 2.6. Non-structural carbohydrate distributions

Plant materials for nonstructural carbohydrate (NSC) analysis were sampled during the course of the experiment, in seven measurement days. Shoots including bark and fine roots were sampled from 3 to 5 saplings per treatment. Following root washing, all plant materials were microwaved for 30 s to stop further enzymatic activity, and then dried at  $80^\circ\text{C}$  for 48 h. Samples were then ground carefully using a ball mill (Retsch, Haan, Germany) at a frequency of  $25 \text{ s}^{-1}$  until tissues turned into fine powder ( $\sim 5 \text{ min}$ ). NSC analyses followed the method by Wong (1990), modified as described in Hoch et al. (2002). Dried roots and shoot powder (8–12 mg) was extracted with 2 mL deionized water at  $100^\circ\text{C}$  for 30 min. An aliquot of each sample extract was taken for the determination of low-molecular weight carbohydrates using invertase (from Baker's yeast) to break sucrose into glucose and fructose. Glucose and fructose were converted into gluconate-6-phosphate using glucose hexokinase and phosphogluconate isomerase (from Baker's yeast). The total amount of formed gluconate-6-phosphate was determined as the increase in  $\text{NADH} + \text{H}^+$  using a photometer (HR 700; Hamilton, Reno, NE, USA). For NSC determination, an aliquot of the remaining extract was incubated at  $4^\circ\text{C}$  for 15 h with amyloglucosidase from *Aspergillus niger* to break starch into glucose. Nonstructural carbohydrate was determined as the total amount of glucose as described above. Starch content was calculated as total NSC minus free sugars. All concentrations were calculated on a% d.m. basis. All Enzymes were purchased from Sigma-Aldrich (St. Louis, MO, USA).

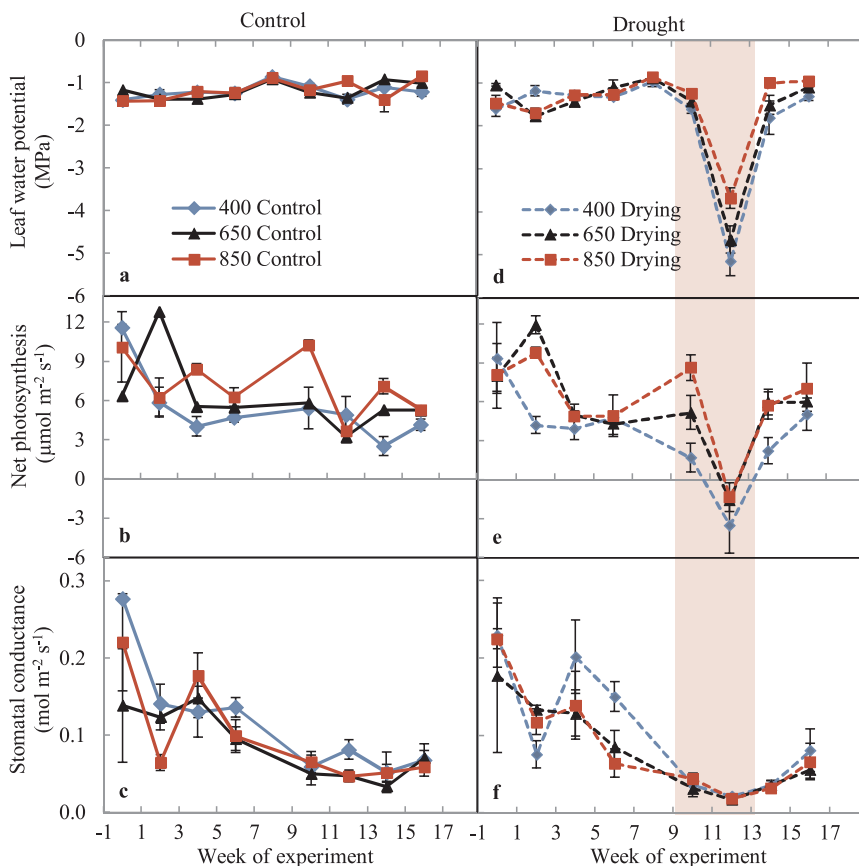
## 2.7. Supplementary experiments

The experiment described in this paper was part of a larger research program on lemon drought resistance, including measurements on mature trees in an orchard, and a second greenhouse experiment on 4-year-old trees grown in 35 L pots. The orchard experiment took place in 2016 in a 20-year-old experimental orchard in the Agricultural Research Organization campus in Rishon LeZion, 10 km SE of Tel Aviv. Five trees were supplied with deficit irrigation during the dry season, which was withheld from five neighboring trees. The second greenhouse experiment started in December 2016 using the same greenhouse rooms in Gilat research station. Five trees were grown at  $[\text{CO}_2]$  of 400 ppm and other five were grown at  $[\text{CO}_2]$  of 850 ppm.

After 2 months of  $\text{CO}_2$  treatment (400 or 850 ppm) trees were moved to a regular greenhouse, where a 2-month dry-down cycle was performed. Leaf water potential and gas exchange measurements were performed as described above in 6 campaigns during 2016 in the orchard and at two time-points (end of  $\text{CO}_2$  treatment and end of drought) in the greenhouse.

## 2.8. Statistical analysis

Seasonal leaf gas exchange, water potential, soil water content, and



**Fig. 1.** Leaf physiology changes in lemon saplings in course of the experiment. Shaded area denotes the 4 weeks of drought imposed on thirty of the sixty saplings (dashed lines). Error bars denote the standard error of the mean ( $n = 4$ ). See Tables 1 and 2 for statistical analysis.

plant growth data were each analyzed using a repeated-measurements analysis of variance (ANOVA) with  $[\text{CO}_2]$  and drought treatments as fixed factors, assuming that the variance within subjects (i.e., the saplings) was homogeneous. For all analyses, there were three-four replicates of all treatments. Effects of  $[\text{CO}_2]$  and drought treatments and their interaction were tested statistically at the beginning, end of the drought period, and after recovery time using two-way ANOVA. When effects of treatments were statistically significant, differences among groups were post hoc tested with Tukey's HSD test.

### 3. Results

#### 3.1. Leaf-scale responses to elevated $\text{CO}_2$ and drought

Leaf water potential was stable around  $-1.4$  MPa across control (irrigated) trees, dropping sharply under drought to  $-3.7$ ,  $-4.6$ , and  $-5.1$  MPa in trees grown at 850, 650, and 400 ppm  $\text{CO}_2$ , respectively (Fig. 1a). The water stress was significantly less pronounced under elevated  $[\text{CO}_2]$ , at 850 ppm, than at ambient  $\text{CO}_2$  ( $P < 0.001$ , Table 1). Following re-watering, trees with elevated  $[\text{CO}_2]$  recovered earlier than at ambient  $[\text{CO}_2]$ , but values were overall not significantly different after four weeks of recovery period (Fig. 1d).

Control trees grown under ambient  $[\text{CO}_2]$  showed an average photosynthesis rate of  $6 \pm 2 \mu\text{mol m}^{-2} \text{s}^{-1}$ , which was 15% and 25% lower than that of the control trees grown at 650 and 850 ppm, respectively (Fig. 1b). Under drought conditions, trees exhibited a negative C balance of  $-3.5$  and  $-1.3 \mu\text{mol m}^{-2} \text{s}^{-1}$  at ambient and both elevated  $\text{CO}_2$  levels, respectively, significantly different than their control neighbors ( $P < 0.001$ ) (Fig. 1b, Table 1); and specifically during late drought (Table 2). Upon re-watering, photosynthesis recovered in all trees, but more so in trees grown under elevated  $\text{CO}_2$  (Fig. 1b).

In contrast to the photosynthesis response to  $\text{CO}_2$ , stomatal

conductance ( $g_s$ ) rather decreased with elevated  $[\text{CO}_2]$ , with uniform stomatal closure under drought. Control trees grown under ambient  $[\text{CO}_2]$  exhibited a  $g_s$  of  $0.14 \text{ mol m}^{-2} \text{s}^{-1}$  on average (Fig. 1c), showing a decreasing trend toward the end of the experiment when air humidity decreased (see 2.1.). With respect to the ambient  $[\text{CO}_2]$ ,  $g_s$  was significantly lower in elevated than ambient  $[\text{CO}_2]$  trees, on average by 25% at 650 ppm and by 11% at 850 ppm in control trees (Fig. 1c, Tables 1 and 2). Upon re-watering, stomatal opening recovered in all trees, as expected more so in trees grown under ambient  $[\text{CO}_2]$  (Fig. 1c, f).

The observed stomatal downregulation was further investigated in week 2 by exposing leaves of trees grown at each  $[\text{CO}_2]$  to multiple  $\text{CO}_2$  concentrations between 0 and 850 ppm and measuring their photosynthesis rate (Fig. 2) and stomatal conductance (Fig. S2). Trees grown at ambient  $\text{CO}_2$  showed the expected polynomial response, with major increases in photosynthesis as  $[\text{CO}_2]$  increased from 200 to 600 ppm ( $P_n = -2 \times 10^{-5} [\text{CO}_2]^2 + 0.04 [\text{CO}_2] + 4.4$ ;  $R^2 = 0.95$ ), tipping off at 800 ppm. In contrast, trees grown at elevated  $\text{CO}_2$  were less responsive to changes in  $\text{CO}_2$  ( $P_n = 0.015 [\text{CO}_2] - 3.8$ ;  $R^2 = 0.78$  at 650 ppm; and  $P_n = 0.011 [\text{CO}_2] - 2.0$ ;  $R^2 = 0.95$  at 850 ppm). Further, we fitted  $A/C_i$  curves to the Sharkey et al. (2007) equations and computed the maximum rate of carboxylation ( $V_{\text{cmax}}$ ). Saplings grown at 650 and 850 ppm had higher  $V_{\text{cmax}}$  (65 and  $56 \mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively) than saplings grown at 400 ppm ( $43 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), indicating no down-regulation of photosynthesis. In terms of  $g_s$ , there was reduced sensitivity to  $\text{CO}_2$  in leaves of trees grown under 850 ppm, and lower  $g_s$  in leaves of trees grown under 650 vs. 400 ppm at an immediate  $\text{CO}_2$  level lower than 300 ppm. Interestingly, at 400 and 500 ppm, leaves of trees grown under 650 ppm had moderately higher  $g_s$  than those grown under 400 ppm.

#### 3.2. Elevated $\text{CO}_2$ and drought effects on plant water balance

Control saplings grown under ambient  $[\text{CO}_2]$  exhibited a



**Table 1**

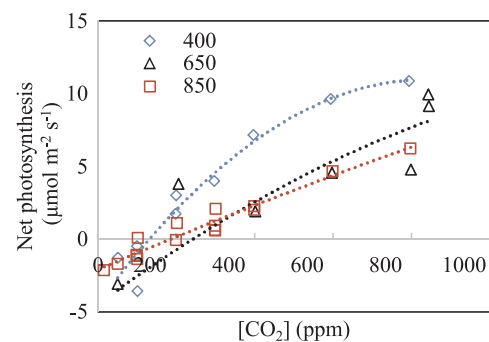
ANOVA Results for the effects of drought, [CO<sub>2</sub>], and their interaction on sapling photosynthesis (P<sub>n</sub>), stomatal conductance (g<sub>s</sub>), transpiration (Tr), leaf water potential (LWP), and pot soil water content (SWC) at 2 and 4 weeks following holding of irrigation and 4 weeks following re-irrigation. Significant effects at the 0.05 significance level are in **boldface**.

2 weeks under drought	P <sub>n</sub>		g <sub>s</sub>		T <sub>r</sub>		WP		SWC	
	F value	P	F value	P	F value	P	F value	P	F value	P
CO <sub>2</sub>	26.1	< 0.0001	4.13	0.05	1.3	0.27	3.3	<b>0.08</b>	9.02	<b>0.004</b>
drought	4.68	<b>0.04</b>	3.24	0.08	5.7	<b>0.026</b>	17.7	<b>0.0004</b>	56	< 0.0001
CO <sub>2</sub> *drought	0.95	0.34	1.15	0.29	0.0098	0.92	9.8	<b>0.005</b>	1.31	<b>0.25</b>
4 weeks under drought										
CO <sub>2</sub>	0.13	0.71	10.3	<b>0.0044</b>	6.2	<b>0.02</b>	17.68	<b>0.0004</b>	–	–
drought	39.8	< 0.0001	45.7	< 0.0001	34.4	< 0.0001	324.5	< 0.0001	–	–
CO <sub>2</sub> *drought	2.24	0.15	7.8	<b>0.01</b>	6.12	<b>0.0224</b>	5.4	<b>0.03</b>	–	–
4 weeks re-watering										
CO <sub>2</sub>	1.09	0.35	0.26	0.77	0.32	0.73	11	<b>0.0008</b>	9.02	0.004
drought	1.58	0.22	0.015	0.9	0.0003	0.95	2.1	0.16	0.92	< 0.00001
CO <sub>2</sub> *drought	0.11	0.9	0.32	0.74	0.31	0.73	0.003	1	1.31	0.25

transpiration (Tr) rate of 3 mmol m<sup>-2</sup> s<sup>-1</sup> on average (Fig. 3a), showing a decreasing slope toward the end of the experiment when air humidity decreased (see 2.1.). Tr was decreased by elevated [CO<sub>2</sub>] after six weeks and on throughout the experiment under control conditions, except for week 12 at intermediate [CO<sub>2</sub>] level (average of 34% at intermediate and 15% at high [CO<sub>2</sub>]). The irrigation manipulation equally reduced Tr in all drought trees and increased Tr after re-watering with no significant different between the [CO<sub>2</sub>] (Fig. 3a, Tables 1 and 2).

Daily evapotranspiration (ET) decreased significantly after six weeks of elevated [CO<sub>2</sub>] and throughout the experiment period except for week 12 at intermediate [CO<sub>2</sub>] (Fig. 3b). During the drought period, ET significantly decreased compared to control conditions. Under drought, ET was further significantly decreased at low and intermediate [CO<sub>2</sub>] in comparison to high [CO<sub>2</sub>]. After four weeks of recovery, ET was not significantly different at any [CO<sub>2</sub>] (Fig. 3b and e, Tables 1 and 2).

Under control conditions, soil water content (SWC) was significantly increased after ten weeks and up until the end of the experiment at high [CO<sub>2</sub>] (Fig. 3c). On average, in control conditions elevated [CO<sub>2</sub>] increased SWC by 12% and 18% at intermediate and high [CO<sub>2</sub>] respectively. During drought, the decrease in SWC was less pronounced in ambient compared to elevated [CO<sub>2</sub>] (Fig. 3f). In week 10 SWC was



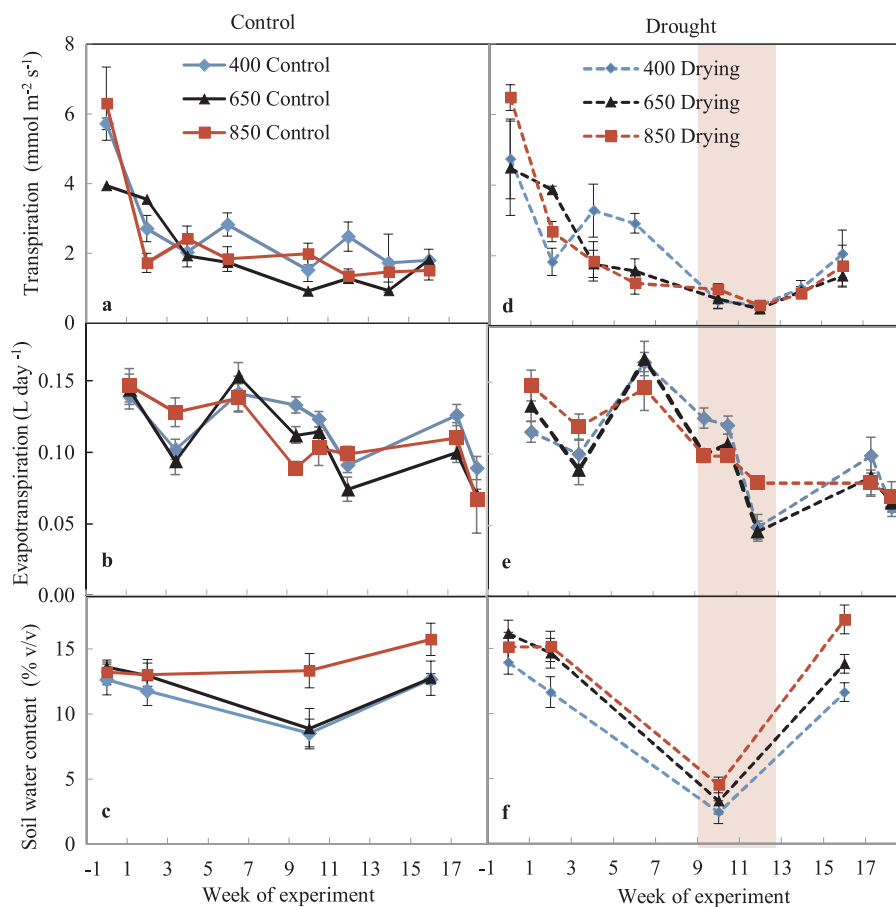
**Fig. 2.** The response of leaf net photosynthesis rate to changes in [CO<sub>2</sub>] in the immediate environment of the sapling, induced by the CO<sub>2</sub> mixer of the gas exchange chamber system.

further significantly decreased at low and intermediate [CO<sub>2</sub>] in comparison with high [CO<sub>2</sub>]. After four weeks of recovery, SWC was still significantly higher at high [CO<sub>2</sub>] compared to intermediate and ambient [CO<sub>2</sub>] (Fig. 3f, Tables 1 and 2).

**Table 2**

ANOVA and mean comparison for the effect of elevated [CO<sub>2</sub>] on sapling photosynthesis (P<sub>n</sub>), stomatal conductance (g<sub>s</sub>), transpiration (T), leaf water potential (LWP), and pot soil water content (SWC) at weeks 6, 10, 12, and 14 of the irrigation experiment (week 16 for recovery SWC). All comparisons are based on values from drought trees, minus the values from control trees (e.g., in the first line: [(400<sub>drought</sub> – 400<sub>control</sub>) – (650<sub>drought</sub> – 650<sub>control</sub>)]). Significant effects at the 0.05 significance level are in **boldface**.

		P <sub>n</sub>		g <sub>s</sub>		T		LWP		SWC	
		Diff	P	Diff	P	Diff	P	Diff	P	Diff	P
Pre-drought (week 6)											
CO <sub>2</sub>	400–650	1.26	0.56	0.024	0.067	0.44	0.67	2.45	0.78	2.26	0.56
	400–850	1.42	<b>0.045</b>	0.049	0.56	0.77	0.53	2.05	0.67	3.42	< <b>0.05</b>
	650–850	0.16	1.09	0.024	0.78	0.26	0.56	0.40	0.986	1.16	1.09
Drought (week 10)											
CO <sub>2</sub>	400–650	3.06	0.10	0.009	0.61	0.66	0.22	3.52	0.07	0.53	0.21
	400–850	2.06	0.20	0.029	0.11	0.12	0.82	4.70	<b>0.026</b>	2.71	0.29
	650–850	1.00	0.60	0.038	<b>0.045</b>	0.78	0.16	1.17	0.52	3.30	0.83
Drought (week 12)											
CO <sub>2</sub>	400–650	3.63	<b>0.026</b>	0.039	0.06	0.04	<b>0.027</b>	1.12	<b>0.036</b>	–	–
	400–850	3.40	<b>0.034</b>	0.041	<b>0.047</b>	0.05	<b>0.022</b>	1.23	<b>0.034</b>	–	–
	650–850	0.30	0.87	0.002	0.99	0.03	0.89	0.02	0.97	–	–
Recovery (week 14)											
CO <sub>2</sub>	400–650	2.05	0.56	0.001	0.87	0.45	0.56	8.12	<b>0.045</b>	0.98	0.76
	400–850	2.25	0.056	0.020	0.98	0.14	0.89	3.30	0.067	1.45	0.45
	650–850	0.25	0.87	0.011	0.678	0.59	0.056	4.82	0.780	1.23	0.67

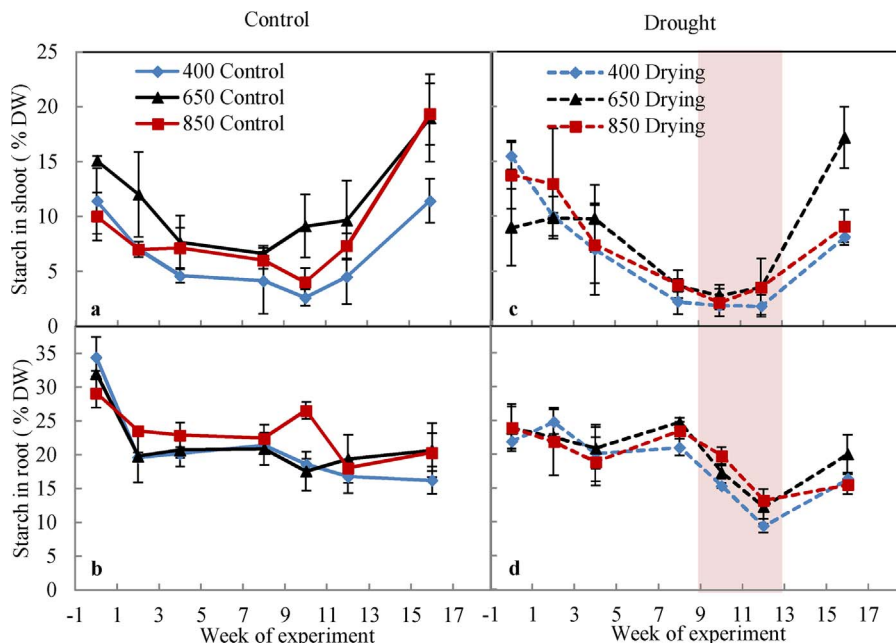


**Fig. 3.** Water balance changes in lemon saplings in course of the experiment. Shaded area denotes the 4 weeks of drought imposed on thirty of the sixty saplings (dashed lines). Error bars denote the standard error of the mean ( $n = 10$  except for  $n = 4$  in (a, d). See Tables 1 and 2 for statistical analysis.

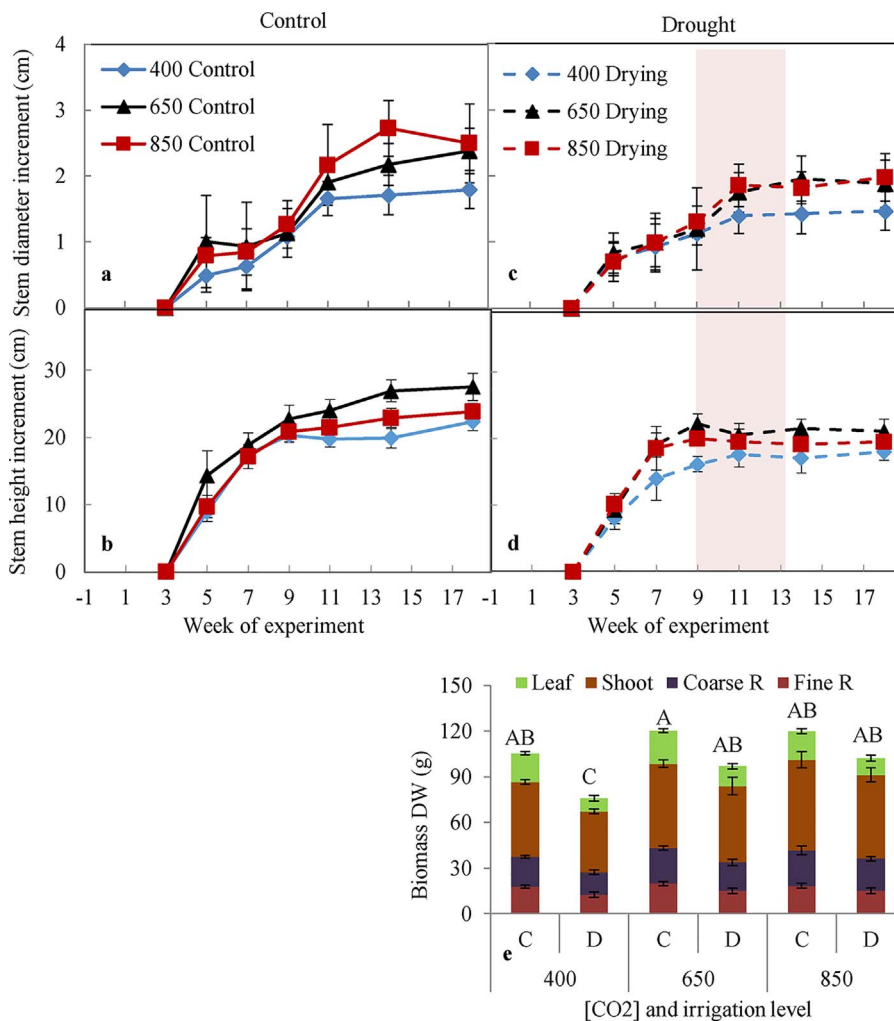
### 3.3. Wood nonstructural carbohydrates under elevated $\text{CO}_2$ and drought

Starch and sugars in root and shoot tissues changed in course of the experiment period, but generally without major differences between  $[\text{CO}_2]$  and drought treatments. Starch levels fluctuated between 1% and 22% d.m. in shoots, and between 9% and 24% d.m. in roots (Fig. 4). Soluble sugar levels fluctuated between 1% and 9% d.m. in shoots, and

between 1% and 8% d.m. in roots (data not shown). Shoot starch decreased to minimum in week 9 to 13, increasing again in week 14 to 18, when shoot sugars decreased dramatically. Root starch decreased gradually through the experiment, while root sugars increased to maximum in week 9 to 13 and decreased sharply in week 14 to 18. These dynamics are probably related to seasonal changes, reflecting the production of new leaves and fine roots in spring. Divergence from these



**Fig. 4.** Changes in shoot and root starch content in lemon saplings in course of the experiment. Shaded area denotes the 4 weeks of drought imposed on thirty of the sixty saplings (dashed lines). Error bars denote the standard error of the mean ( $n = 6$ ).



**Fig. 5.** Growth of lemon saplings in course of the experiment (a–d) and biomass partitioning at the end of the experiment (e). Shaded area denotes the 4 weeks of drought imposed on thirty of the sixty saplings (dashed lines). DW, dry weight; R, roots; C, control; D, drought. Error bars denote the standard error of the mean ( $n = 10$ ). Significant differences in (e) are marked by different letters (Tukey's HSD *t*-test  $p < 0.05$ ). See Table 3 for full statistical analysis.

trends was observed in two instances: (1) Shoot starch of control saplings at the intermediate [CO<sub>2</sub>] increased in week 9 to 13 (from 3% to 9% d.m.), four weeks earlier than in the other saplings (Fig. 4a); and (2) Root starch of control saplings at the high [CO<sub>2</sub>] transiently increased (from 22% to 27% d.m.) in week 9 to 13 (Fig. 4b). Both cases precluded saplings at ambient [CO<sub>2</sub>] or experiencing drought.

### 3.4. Sapling growth under elevated CO<sub>2</sub> and drought

Stem diameter significantly increased over time in control trees grown at any [CO<sub>2</sub>], without significant differences between the different [CO<sub>2</sub>] treatments (Fig. 5a and c). Under drought conditions diameter increment significantly decreased in ambient [CO<sub>2</sub>] trees compared to the elevated [CO<sub>2</sub>] trees. Unlike the diameter increment, the increase in tree height stopped at week 8. Over the eighteen week of the experiment, control plants grown in ambient [CO<sub>2</sub>] had consistently and significantly lower heights (Fig. 5b and d) than that of the plants grown in elevated [CO<sub>2</sub>], in average by 18% at both 650 and 850 ppm (Fig. 5b). Under drought conditions, plants grown under elevated [CO<sub>2</sub>] were 25% taller than ambient [CO<sub>2</sub>] trees (Fig. 5d).

At the end of the experiment, leaf, shoot, coarse root and fine root biomass distribution was quantified. The biomass of control plants grown under elevated [CO<sub>2</sub>] was insignificantly higher for leaves, shoots, coarse roots and fine roots (Fig. 5e and Table 3). Under drought conditions, only fine roots and leaf biomass was significantly decreased compared to control at all [CO<sub>2</sub>], by 30, 25, 20 and 55, 55, 38% of at ambient, intermediate and high [CO<sub>2</sub>] conditions respectively (Fig. 5e and Table 3). Leaf, coarse roots and stem biomass were significantly

decreased under drought by 28, 29 and 30% respectively at ambient compared to high [CO<sub>2</sub>], but not at intermediate level (Fig. 5e and Table 3). Overall, only the trees grown at ambient [CO<sub>2</sub>] and treated with drought had a significantly lower biomass than all the other treatment combinations. The root: shoot ratio was similar across all CO<sub>2</sub> and drought treatments, with investment into root biomass insignificantly increasing with [CO<sub>2</sub>] ( $0.56 \pm 0.01$ ,  $0.57 \pm 0.03$  and  $0.58 \pm 0.03$  at 400, 650, and 850 ppm, respectively). The compensation of elevated CO<sub>2</sub> for drought effects was highly evident at the end of the dry-down cycle (Fig. 6), with full, partial, and zero leaf wilting in drought-exposed saplings growing at 400, 650, and 850 ppm [CO<sub>2</sub>], respectively. Toward the end of the experiment, most saplings had chlorotic leaves in part of their foliage (Fig. 6). At the end of the recovery period, saplings grown in elevated [CO<sub>2</sub>] were fully active, but at ambient conditions, nearly 10% of saplings didn't produce new leaves. Analysis of macro elements in dry leaf tissue of all saplings showed N, P, and K concentrations of 1.15–1.91, 0.17–0.29, and 1.20–1.75 g m<sup>-2</sup>. These values were similar across CO<sub>2</sub> and irrigation treatments, and fall within the observed ranges in healthy leaves. Hence, the chlorotic appearance might be related to micronutrient deficiency, e.g. due to insufficient Mg supply.

### 3.5. Influences of CO<sub>2</sub> and drought on mature lemon trees

As in any greenhouse experiment on saplings, transferability of the observations to mature trees is limited unless supported. Results from two experiments running in parallel to the experiment described above are provided in the Method sections. In 4-year-old lemon trees, 2-

**Table 3**

ANOVA *P*-values for the effect of elevated [CO<sub>2</sub>], drought, and their interaction on tree growth and biomass parameters at the end of the irrigation experiment. Significant effects at the 0.05 significance level are in **boldface**.

Treatment	Stem diameter increment	Stem height increment	Fine root biomass	Coarse root biomass	Stem biomass	Leaf biomass	Leaf area	Total tree biomass	Root: shoot ratio
CO <sub>2</sub>	<b>0.0056</b>	<b>0.0045</b>	0.47	<b>0.018</b>	<b>0.037</b>	0.41	0.3	<b>0.03</b>	0.85
drought	0.236	0.125	<b>0.0002</b>	<b>0.011</b>	0.25	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>0.0003</b>	0.99
CO <sub>2</sub> * drought	0.123	0.06	0.59	0.74	0.25	0.57	0.72	0.24	0.92



**Fig. 6.** Lemon saplings after 4 weeks of drought. The three trees were exposed to the same drought regime.

months drought decreased LWP from  $-0.6$  and  $-1.2$  MPa (at 400 and 850 ppm CO<sub>2</sub>, respectively) to  $-3.8$  MPa; stomatal conductance decreased from  $0.13$  to  $0.04$  mol m<sup>-2</sup> s<sup>-1</sup>; and photosynthesis decreased from  $6.4$  and  $4.0$  μmol m<sup>-2</sup> s<sup>-1</sup> (at 400 and 850 ppm CO<sub>2</sub>, respectively) to  $1.6$  μmol m<sup>-2</sup> s<sup>-1</sup> (Fig. S3). These effects were diminished after the young trees were exposed to the long drought without the CO<sub>2</sub> treatment (unlike in our major experiment). In mature trees in the field, stomatal conductance decreased from  $0.08$  mol m<sup>-2</sup> s<sup>-1</sup> in the wet season to near-zero in the dry season (Fig. S4). At the same time LWP decreased from  $-1.0$  to  $-3.2$  and  $-2.8$  MPa, depending on the drought level.

#### 4. Discussion

Elevated [CO<sub>2</sub>] partially relieved drought stress in young lemon trees. In drought conditions, trees growing in elevated [CO<sub>2</sub>] showed a lesser drop in LWP (Fig. 1a, d) and a slower drop in photosynthesis (Fig. 1b, e) than trees growing in ambient [CO<sub>2</sub>]. Elevated [CO<sub>2</sub>] may compensate for drought stress (decreases in leaf water potential, photosynthesis, and growth) and speed-up recovery by three linked, but distinct mechanisms. In the first mechanism, elevated [CO<sub>2</sub>] causes stomata to close, causing the tree to lose less water, at otherwise the

same or higher carbon supply, both before and during drought, thus causing tree leaves to retain more moisture. The downregulation of stomata to elevated [CO<sub>2</sub>] (Fig. 2 and Fig. S2) can be part of a sink-control over photosynthesis (Körner, 2015), rather than the old paradigm by which photosynthesis drives plant carbon metabolism. The second, related, mechanism acts as tree water-use under elevated [CO<sub>2</sub>] decreases compared to the ambient conditions, thereby retaining more water in the soil (Niklaus et al., 1998). As a result of this plant-soil feedback, trees growing at elevated [CO<sub>2</sub>] may have higher soil water content, which is critical under drying conditions. In the third mechanism, the elevated [CO<sub>2</sub>] allows the tree to store more starch (Körner et al., 2005), allowing it to survive for a longer time period after stomata are kept closed during drought.

In this experiment, the first mechanism was clearly demonstrated. The stomata have been shown to open less in elevated [CO<sub>2</sub>] during the weeks leading up to the drought (Fig. 1c, f). In turn, this led to less water being used by the trees in elevated [CO<sub>2</sub>] (Fig. 3). Across all treatments, leaf stomatal conductance and transpiration eventually fell to nearly 0 during the drought. However, the water saved over the course of the pre-drought period and the beginning of the drought in the elevated [CO<sub>2</sub>] treatments was evident by higher soil water content at [CO<sub>2</sub>] of 850 ppm (Fig. 3c, Table 2, pre-drought). SWC of plants under 850 ppm [CO<sub>2</sub>] was typically higher than in other plants, but the difference was significant only before the drought. The faster recovery of most parameters at elevated [CO<sub>2</sub>] indicates its role in drought recovery, in addition to drought resistance. It was suggested that the relative increase in photosynthesis at elevated CO<sub>2</sub> should be larger under drought than under non-drought conditions (Franks et al., 2013). Here we showed significant photosynthetic enhancement during early drought, and significant drought-induced reduction in Pn (Table 1), although the CO<sub>2</sub>\*drought interaction was not significant. Also, the observed stomatal downregulation is in line with the theory that many plants maintain a constant relative gradient for CO<sub>2</sub> diffusion into the leaf (Franks et al., 2013). Roy et al. (2016) demonstrated that grassland stomatal conductance was reduced and water-use efficiency and soil water status were increased. Similarly, Ghini et al. (2015), Tognetti et al. (2001), Centritto et al. (1999a,b), Centritto et al. (2002) and Fitzgerald et al. (2016) showed similar patterns for crops (coffee, olives, cherry, peach, and wheat, respectively). On the other hand, Moutinho-Pereira et al. (2015) and Gray et al. (2016) did not find any significant effects of elevated CO<sub>2</sub> on plant drought resistance. Potentially, the reduced stomatal sensitivity to elevated [CO<sub>2</sub>] (Fig. 2) could lead to impaired stomatal closure (Heath and Kerstiens 1997; Haworth et al., 2016), but not in our case. Our observations extend on previous reports on elevated [CO<sub>2</sub>] effects on fruit trees (Centritto et al., 1999a,b; Centritto et al., 2002; Goodfellow et al., 1997; Tognetti et al., 2001; Moutinho-Pereira et al., 2015; Roy et al., 2016).

The third mechanism was less verifiable from the data in this experiment. Although starch increased at elevated [CO<sub>2</sub>] in the weeks preceding the drought, a few days into the drought there was no significant difference in the starch content between the elevated and ambient CO<sub>2</sub> treatments (Fig. 4a, c). Therefore, it is hard to argue that the milder drought stress under elevated [CO<sub>2</sub>] was related to increased starch availability (Hartmann and Trumbore, 2016). Probably, the higher C uptake of elevated [CO<sub>2</sub>] trees under drought was counter-



balanced by the higher C demand for growth relative to ambient  $[\text{CO}_2]$  trees (Fig. 5), resulting in no difference of the C reserve pool between the  $\text{CO}_2$  treatments. This is possible, since growth and storage processes can compete over available plant C (Wiley and Helliker, 2012).

The complexity of tree C metabolism meant that responses to the level of  $\text{CO}_2$  were monotonous at times, but not always. For example, upon initiation of the drought stress, water loss rate was roughly 0.12, 0.11, and  $0.11 \text{ L d}^{-1}$  at 400, 650, and 850 ppm  $[\text{CO}_2]$ , respectively (Fig. 3b); but this hierarchy changed as drought progressed: water loss rate at 850 ppm was still high whereas at 400 and 650 ppm it dropped sharply (Fig. 3e). This could be explained by the higher soil water content at the higher  $[\text{CO}_2]$  (Niklaus et al., 1998). Our measurements also reveal changes in C allocation: transient increases in tissue starch under elevated  $[\text{CO}_2]$  were observed in the shoot at 650 ppm, and in roots at 850 ppm (Fig. 4). And interestingly, the small growth increment was similar across the two  $[\text{CO}_2]$  levels (Fig. 5), and differences in C supply were inconsistent or absent (Fig. 1b, e). In summary, the increase in  $[\text{CO}_2]$  from 650 to 850 ppm was usually not additive (compare the treatment differences in Table 2), suggesting that even under near-optimal conditions (e.g. temperature, nutrition), young trees can be  $\text{CO}_2$ -saturated at 650 ppm or less, as argued earlier for trees in general (Körner, 2003; Körner et al., 2005).

How relevant are these observations to long-term effects on mature trees in the field? Our experiment took place in Gilat Research Center at the heart of the Israeli “citrus belt” and using the same sandy loam soil. Measurements in 4-year-old lemon trees confirmed our observations in saplings, showing higher photosynthesis at rather similar stomatal conductance and milder LWP at elevated vs. ambient  $\text{CO}_2$  (Fig. S3). Our lemon saplings showed similar behavior with the 4-year-old and the 20-year-old lemon trees (Fig. S4), albeit with mildly higher  $g_s$  and more negative LWP. Sapling gas exchange values were also comparable to those reported previously for lemon trees in orchards (Ortuño et al., 2006; Rodríguez-Gamir et al., 2010). In this respect, 1-year old, 1-m high saplings are comparable in most physiological aspects to mature trees in some species (Klein et al., 2011) but not in all species (Bond, 2000).

Due to the time limitation of our experimental system, and given the experimental setting (including space and tree size limitations), we used four months of exposure to elevated  $\text{CO}_2$  as representation of a longer-term  $\text{CO}_2$  enrichment. Free-air  $\text{CO}_2$  enrichment experiments have already indicated the transient nature of initial responses (e.g. Oren et al., 2001; Körner et al., 2005; Klein et al., 2016a). However those observations were from forests, which are much more complex tree communities than orchards, and are far more limited in terms of access to light, water, and minerals. Despite of the observed reduction in stomatal conductance in response to elevated  $\text{CO}_2$ , stomata appear to be less limiting to photosynthesis. This result was consistent with previous findings in olive leaves after 7 months of FACE (Tognetti et al., 1998), in mango leaves (Bunce, 1992) and sour oranges after 2.5 years of FACE (Idso et al., 1991). A less negative LWP at elevated  $\text{CO}_2$  observed in our study was in line with some studies (Rogers et al., 1984; Grant et al., 1995; Huxman et al., 1999; Domec et al., 2017) but not with others (Johnson et al., 1993; Townend, 1993; Walker et al., 1997; Tognetti et al., 2000). Similarly, in a FACE setting, Ellsworth (1999) and Domec et al. (2009) observed little or no effect of elevated  $\text{CO}_2$  on LWP and soil moisture in field-grown *Pinus taeda*.

In contrast with the milder conditions in the greenhouse vs. the orchard, the dry-down cycle we applied was as extreme, if not harsher, than a dry spell occurring naturally, or any deficit irrigation practice in a lemon orchard. The water potential values measured here (Fig. 1a) were substantially lower than previously reported for lemon trees in orchards (Nadler et al., 2003; Ortuño et al., 2006; García-Orellana et al., 2007; Raveh, 2008; Ortuño et al., 2009; Rodríguez-Gamir et al., 2010). The confined pot volume ensured the rapid development of water stress throughout the plant, further affecting aboveground tissues. Special attention was given to evade the development of a pot

binding effect (see 2.1.) and we are confident that root development was not limited in this experiment. On this background, it is important to note that elevated  $\text{CO}_2$  effects were still measurable in spite of the extreme drought. Evidently, all drought-subjected trees closed stomata, suffered LWP  $\leq 3.5 \text{ MPa}$ , a negative C balance, and reductions in shoot starch. But the elevated  $\text{CO}_2$  compensated in part for these responses, which are further indicated by higher  $V_{\text{cmax}}$ . There are at least two reasons to believe that the observed compensation would persist, or even intensify, in a high- $\text{CO}_2$  orchard: (1) the increased photosynthesis at elevated  $\text{CO}_2$  might translate into higher investment in belowground C sinks, and hence proliferation of the root systems, in turn further increasing the drought resistance of high- $\text{CO}_2$  trees; (2) Tree age can also change how elevated  $\text{CO}_2$  affects a tree during a drought: Older trees may have more capacity to store starch, causing the effect of elevated  $\text{CO}_2$  on starch storage to be bigger than observed here.

In conclusion, our results indicate that in a high- $\text{CO}_2$  future, fruit trees in orchards, and perhaps also forest trees growing in low-density stands on nutrient-rich soil will (1) use less water, at otherwise similar conditions, than today; (2) have higher water availability when drought hits; and (3) have higher carbon storage to survive through drought. Although these three effects are positive in regards to tree survival, future risks to orchards and forests may still increase due to anthropogenic climate change.

## Conflict of interest

The authors declare no conflict of interest.

## Author contributions

IP, MH, and TK performed the measurements with help from GH, YW and ER; UY designed the  $\text{CO}_2$  system; TK designed the study; IP, MH, and TK wrote the paper with help from GH, UY and ER.

## Acknowledgments

TK wishes to thank the Merle S. Cahn Foundation and the Monroe and Marjorie Burk Fund for Alternative Energy Studies; Mr. and Mrs. Norman Reiser, together with the Weizmann Center for New Scientists; and the Edith & Nathan Goldberg Career Development Chair. GH thanks Sandra Schmid for her expertise with the NSC analysis.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.envexpbot.2018.01.004>.

## References

- Ainsworth, E.A., Davey, P.A., Bernacchi, C.J., Dermody, O.C., Heaton, E.A., Moore, D.J., Morgan, P.B., Naidu, S.L., Yoo Ra, H.S., Zhu, X.G., Curtis, P.S., 2002. A meta-analysis of elevated  $[\text{CO}_2]$  effects on soybean (glycine max) physiology, growth and yield. *Global Change Biol.* 8, 695–709.
- Beerling, D.J., Heath, J., Woodward, F.I., Mansfield, T.A., 1996. Drought-  $\text{CO}_2$  interactions in trees: observations and mechanisms. *New Phytol.* 134, 235–242.
- Bond, B.J., 2000. Age-related changes in photosynthesis of woody plants. *Trends Plant Sci.* 5, 349–353.
- Bunce, J.A., 1992. Stomatal conductance, photosynthesis and respiration of temperate deciduous tree seedlings grown outdoors at an elevated concentration of carbon dioxide. *Plant Cell Environ.* 15, 541–549.
- Centritto, M., Lee, H.S., Jarvis, P.G., 1999a. Interactive effects of elevated  $[\text{CO}_2]$  and drought on cherry (*Prunus avium*) seedlings I. Growth, whole-plant water use efficiency and water loss. *New Phytol.* 141, 129–140.
- Centritto, M., Magnani, F., Lee, H.S., Jarvis, P.G., 1999b. Interactive effects of elevated  $[\text{CO}_2]$  and drought on cherry (*Prunus avium*) seedlings II: photosynthetic capacity and water relations. *New Phytol.* 141, 141–153.
- Centritto, M., Lucas, M.E., Jarvis, P.G., 2002. Gas exchange, biomass, whole-plant water-use efficiency and water uptake of peach (*Prunus persica*) seedlings in response to elevated carbon dioxide concentration and water availability. *Tree Physiol.* 22, 699–706.
- Culotta, E., 1995. Will plants profits from high  $\text{CO}_2$ . *Science* 268, 5211.

- De Gelder, A., Heuvelink, E., Opdam, J.J.G., 2005. Tomato yield in a closed greenhouse and comparison with simulated yields in closed and conventional greenhouses. *Acta Hort.* 691, 549–552.
- Domec, J.C., Palmroth, S., Ward, E., Maier, C.A., Thérézien, M., Oren, R.A., 2009. Acclimation of leaf hydraulic conductance and stomatal conductance of *Pinus taeda* (loblolly pine) to long-term growth in elevated CO<sub>2</sub> (free-air CO<sub>2</sub> enrichment) and N-fertilization. *Plant Cell Environ.* 32, 1500–1512.
- Domec, J.C., Smith, D.D., McCulloh, K.A., 2017. A synthesis of the effects of atmospheric carbon dioxide enrichment on plant hydraulics: implications for whole-plant water use efficiency and resistance to drought. *Plant Cell Environ.* 40, 921–937.
- Duan, H., Duursma, R.A., Huang, G., Smith, R.A., Choat, B., Ogrady, A.P., Tissue, D.T., 2014. Elevated [CO<sub>2</sub>] does not ameliorate the negative effects of elevated temperature on drought-induced mortality in *Eucalyptus radiata* seedlings. *Plant Cell Environ.* 37, 1598–1613.
- Ellsworth, D.S., 1999. CO<sub>2</sub> enrichment in a maturing pine forest: are CO<sub>2</sub> exchange and water status in the canopy affected? *Plant Cell Environ.* 22, 461–472.
- Fitzgerald, G.J., Tausz, M., O'Leary, G., Mollah, M.R., Tausz-Posch, S., Seneweera, S., Norton, R.M., 2016. Elevated atmospheric [CO<sub>2</sub>] can dramatically increase wheat yields in semi-arid environments and buffer against heat waves. *Glob. Change Biol.* 22, 2269–2284.
- Franks, P.J., Adams, M.A., Amthor, J.S., Barbour, M.M., Berry, J.A., Ellsworth, D.S., Norby, R.J., 2013. Sensitivity of plants to changing atmospheric CO<sub>2</sub> concentration: from the geological past to the next century. *New Phytol.* 197 (4), 1077–1094.
- Friedman, S.P., 2016. Evaluating the role of water availability in determining the yield–plant population density relationship. *Soil Sci. Soc. Am. J.* 80, 563–578.
- García-Orellana, Y., Ruiz-Sánchez, M.C., Alarcón, J.J., Conejero, W., Ortuño, M.F., Nicolás, E., Torrecillas, A., 2007. Preliminary assessment of the feasibility of using maximum daily trunk shrinkage for irrigation scheduling in lemon trees. *Agric. Water Manage.* 89, 167–171.
- Ghini, R., Torre-Neto, A., Dentzien, A.F., Guerreiro-Filho, O., Iost, R., Patrício, F.R., ... Da Matta, F.M., 2015. Coffee growth, pest and yield responses to free-air CO<sub>2</sub> enrichment. *Clim. Change* 132, 307–320.
- Goodfellow, J., Eamus, D., Duff, G., 1997. Diurnal and seasonal changes in the impact of CO<sub>2</sub> enrichment on assimilation, stomatal conductance and growth in a long-term study of *Mangifera indica* in the wet–dry tropics of Australia. *Tree Physiol.* 17, 291–299.
- Grant, R., Garcia, R., Pinter, J.P., Hunsaker, D., Wall, G., Kimball, B., LaMorte, R., 1995. Interaction between atmospheric CO<sub>2</sub> concentration and water deficit on gas exchange and crop growth: testing of ecosystems with data from the free air CO<sub>2</sub> enrichment (FACE) experiment. *Global Change Biol.* 1, 443–454.
- Gray, S.B., Dermody, O., Klein, S.P., Locke, A.M., McGrath, J.M., Paul, R.E., Ainsworth, E.A., 2016. Intensifying drought eliminates the expected benefits of elevated carbon dioxide for soybean. *Nat. Plants* 2, 16132.
- Guehl, J.M., Picon, C., Aussenac, G., Gross, P., 1994. Interactive effects of elevated CO<sub>2</sub> and soil drought on growth and transpiration efficiency and its determinants in two European forest tree species. *Tree Physiol.* 14, 707–724.
- Hartmann, H., Trumbore, S., 2016. Understanding the roles of nonstructural carbohydrates in forest trees—from what we can measure to what we want to know. *New Phytol.* 211, 386–403.
- Haworth, M., Killi, D., Materassi, A., Raschi, A., Centritto, M., 2016. Impaired stomatal control is associated with reduced photosynthetic physiology in crop species grown at elevated [CO<sub>2</sub>]. *Front. Plant Sci.* 7.
- Heath, J., Kerstiens, G., 1997. Effects of elevated CO<sub>2</sub> on leaf gas exchange in beech and oak at two levels of nutrient supply: consequences for sensitivity to drought in beech. *Plant Cell Environ.* 20, 57–67.
- Hoch, G., Popp, M., Körner, C., 2002. Altitudinal increase of mobile carbon pools in *Pinus cembra* suggests sink limitation of growth at the Swiss treeline. *Oikos* 98, 361–374.
- Huxman, T.E., Hamerlynck, E.P., Smith, S.D., 1999. Reproductive allocation and seed production in *Bromus madritensis* ssp. *rubens* at elevated atmospheric CO<sub>2</sub>. *Funct. Ecol.* 13, 769–777.
- Idso, S.B., Kimball, B.A., Allen, S.G., 1991. Net photosynthesis of sour orange trees maintained in atmospheres of ambient and elevated CO<sub>2</sub> concentration. *Agric. For. Meteorol.* 54, 95–101.
- Johnson, H.B., Polley, H.W., Mayeux, H.S., 1993. Increasing CO<sub>2</sub> and plant–plant interactions: effects on natural vegetation. CO<sub>2</sub> and Biosphere. Springer, Netherlands, pp. 157–170.
- Jones, M.B., Donnelly, A., 2004. Carbon sequestration in temperate grassland ecosystems and the influence of management climate and elevated CO<sub>2</sub>. *New Phytol.* 164, 423–439.
- Körner, C., Asshoff, R., Bignucolo, O., Hättenschwiler, S., Keel, S.G., Peláez-Ried, S., Zotz, G., 2005. Carbon flux and growth in mature deciduous forest trees exposed to elevated CO<sub>2</sub>. *Science* 309, 1360–1362.
- Körner, C., 2003. Carbon limitation in trees. *J. Ecol.* 91, 4–17.
- Körner, C., 2015. Paradigm shift in plant growth control. *Curr. Opin. Plant Biol.* 25, 107–114.
- Keenan, T.F., Hollinger, D.Y., Bohrer, G., Dragoni, D., Munger, J.W., Schmid, H.P., Richardson, A.D., 2013. Increase in forest water-use efficiency as atmospheric carbon dioxide concentrations rise. *Nature* 499, 324–327.
- Klein, T., Hoch, G., 2015. Tree carbon allocation dynamics determined using a carbon mass balance approach. *New Phytol.* 205 (1), 147–159.
- Klein, T., Cohen, S., Yakir, D., 2011. Hydraulic adjustments underlying drought resistance of *Pinus halepensis*. *Tree Physiol.* 31, 637–648.
- Klein, T., Bader, M.K.F., Leuzinger, S., Mildner, M., Schleppei, P., Siegwolf, R.T., Körner, C., 2016a. Growth and carbon relations of mature *Picea abies* trees under 5 years of free-air CO<sub>2</sub> enrichment. *J. Ecol.* 104, 1720–1733.
- Klein, T., Rotenberg, E., Tatarinov, F., Yakir, D., 2016b. Association between sap flow-derived and eddy covariance-derived measurements of forest canopy CO<sub>2</sub> uptake. *New Phytol.* 209, 436–446.
- Klein, T., 2015. Drought-induced tree mortality: from discrete observations to comprehensive research. *Tree Physiol.* 35, 225–228.
- Li, Y., Xu, H., Cohen, S., 2005. Long term hydraulic acclimation to soil texture and radiation load in cotton. *Plant Cell Environ.* 28, 492–499.
- Liang, N., Maruyama, K., 1995. Interactive effects of CO<sub>2</sub> enrichment and drought stress on gas exchange and water-use efficiency in *Alnus firma*. *Environ. Exp. Bot.* 35, 353–361.
- Martin, C.A., Stutz, J.C., Kimball, B.A., Idso, S.B., Akey, D.H., 1995. Growth and topological changes of *Citrus limon* (L.) burm. f. *Eureka* in response to high temperatures and elevated atmospheric carbon dioxide. *J. Am. Soc. Hortic. Sci.* 120, 1025–1031.
- Maseyk, K., Grunzweig, J.M., Rotenberg, E., Yakir, D., 2008. Respiration acclimation contributes to high carbon-use efficiency in a seasonally dry pine forest. *Global Change Biol.* 14, 1553–1567.
- Moutinho-Pereira, J., Gonçalves, B., Bacelar, E., Cunha, J.B., Countinho, J., Correia, C.M., 2015. Effects of elevated CO<sub>2</sub> on grapevine (*Vitis vinifera* L.): physiological and yield attributes. *Vitis-J. Grapevine Res.* 48, 159.
- Nadler, A., Raveh, E., Yermiyahu, U., Green, S.R., 2003. Evaluation of TDR use to monitor water content in stem of lemon trees and soil and their response to water stress. *Soil Sci. Soc. Am. J.* 67, 437–448.
- Niklaus, P.A., Spinnler, D., Körner, C., 1998. Soil moisture dynamics of calcareous grassland under elevated CO<sub>2</sub>. *Oecologia* 117, 201–208.
- Obrien, M.J., Leuzinger, S., Philipson, C.D., Tay, J., Hector, A., 2014. Drought survival of tropical tree seedlings enhanced by non-structural carbohydrate levels. *Nat. Clim. Change* 4, 710–714.
- Oren, R., Ellsworth, D.S., Johnsen, K.H., Phillips, N., Ewers, B.E., Maier, C., Katul, G.G., 2001. Soil fertility limits carbon sequestration by forest ecosystems in a CO<sub>2</sub>-enriched atmosphere. *Nature* 411, 469–472.
- Ortuño, M.F., García-Orellana, Y., Conejero, W., Ruiz-Sánchez, M.C., Mounzer, O., Alarcón, J.J., Torrecillas, A., 2006. Relationships between climatic variables and sap flow, stem water potential and maximum daily trunk shrinkage in lemon trees. *Plant Soil* 279, 229–242.
- Ortuño, M.F., Brito, J.J., García-Orellana, Y., Conejero, W., Torrecillas, A., 2009. Maximum daily trunk shrinkage and stem water potential reference equations for irrigation scheduling of lemon trees. *Irrig. Sci.* 27, 121.
- Paudel, I., Cohen, S., Shaviv, A., Bar-Tal, A., Bernstein, N., Heuer, B., Ephraim, J., 2016. Impact of treated wastewater on growth, respiration and hydraulic conductivity of citrus root systems in light and heavy soils. *Tree Physiol.* 36, 770–785.
- Poorter, H., Böhler, J., van Dusschoten, D., Climent, J., Postman, J.A., 2012. Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. *Funct. Plant Biol.* 39, 839–850.
- Raveh, E., 2006. Partial root-zone drought as a possible replacement for 'Verdelli' practice in lemon production. V International Symposium on Irrigation of Horticultural Crops 792, 537–541.
- Raveh, E., 2008. Partial root-zone drying as a possible replacement for 'Verdelli' practice in lemon production. *Acta Hort.* 792, 537–541.
- Reyer, C.P., Leuzinger, S., Rammig, A., Wolf, A., Bartholomeus, R.P., Bonfante, A., Klein, T., 2013. A plant's perspective of extremes: terrestrial plant responses to changing climatic variability. *Global Change Biol.* 19, 75–89.
- Robredo, A., Pérez-López, U., de la Maza, H.S., González-Moro, B., Lacuesta, M., Mena-Petite, A., Muñoz-Rueda, A., 2007. Elevated CO<sub>2</sub> alleviates the impact of drought on barley improving water status by lowering stomatal conductance and delaying its effects on photosynthesis. *Environ. Exp. Bot.* 59, 252–263.
- Rodríguez-Gamir, J., Intrigliolo, D.S., Primo-Millo, E., Forner-Giner, M., 2010. Relationships between xylem anatomy, root hydraulic conductivity, leaf/root ratio and transpiration in citrus trees on different rootstocks. *Physiol. Plant* 139, 159–169.
- Rogers, H.H., Sionit, N., Cure, J.D., Smith, J.M., Bingham, G.E., 1984. Influence of elevated carbon dioxide on water relations of soybeans. *Plant Physiol.* 74, 233–238.
- Roy, J., Picon-Cochard, C., Augusti, A., Benot, M.L., Thiery, L., Darsonville, O., Landais, D., Piel, C., Defossez, M., Devidal, S., Escape, C., 2016. Elevated CO<sub>2</sub> maintains grassland net carbon uptake under a future heat and drought extreme. *Proc. Natl. Acad. Sci.* 113, 6224–6229.
- Sarris, D., Christodoulakis, D., Körner, C., 2007. Recent decline in precipitation and tree growth in the eastern Mediterranean. *Global Change Biol.* 13, 1187–1200.
- Savage, J.A., Clearwater, M.J., Haines, D.F., Klein, T., Mencuccini, M., Sevanto, S., Zhang, C., 2016. Allocation, stress tolerance and carbon transport in plants: how does phloem physiology affect plant ecology? *Plant Cell Environ.* 39, 709–725.
- Scott, V., Gilfillan, S., Markusson, N., Chalmers, H., Haszeldine, R.S., 2013. Last chance for carbon capture and storage. *Nat. Clim. Change* 3, 105.
- Sharkey, T.D., Bernacchi, C.J., Farquhar, G.D., Singas, E.L., 2007. Fitting photosynthetic carbon dioxide response curves for C3 leaves. *Plant Cell Environ.* 30, 1035–1040.
- Sholtis, J.D., Gunderson, C.A., Norby, R.J., Tissue, D.T., 2004. Persistent stimulation of photosynthesis by elevated CO<sub>2</sub> in a sweetgum (*Liquidambar styraciflua*) forest stand. *New Phytol.* 162, 343–354.
- Snell, F.D., Snell, C.T., 1949. Colorimetric methods analysis including some turbidimetric and nephelometric methods. Inorganic, vol. 2 Ven Nostrand, Toronto.
- Swann, A.L., Hoffman, F.M., Koven, C.D., Randerson, J.T., 2016. Plant responses to increasing CO<sub>2</sub> reduce estimates of climate impacts on drought severity. *Proc. Natl. Acad. Sci.* 113, 10019–10024.
- Tognetti, R., Longobucco, A., Miglietta, F., Raschi, A., 1998. Transpiration and stomatal behaviour of *Quercus ilex* plants during the summer in a Mediterranean carbon dioxide spring. *Plant Cell Environ.* 21, 613–622.
- Tognetti, R., Cherubini, P., Innes, J.L., 2000. Comparative stem-growth rates of Mediterranean trees under background and naturally enhanced ambient CO<sub>2</sub>

- concentrations. *New Phytol.* 146, 59–74.
- Tognetti, R., Sebastiani, L., Vitagliano, C., Raschi, A., Minnocci, A., 2001. Responses of two olive tree (*Olea europaea* L.) cultivars to elevated CO<sub>2</sub> concentration in the field. *Photosynthetica* 39, 403–410.
- Townend, J., 1993. Effects of elevated carbon dioxide and drought on the growth and physiology of clonal Sitka spruce plants (*Picea sitchensis* (Bong.) Carr.). *Tree Physiol.* 13, 389–399.
- Tschaplinski, T.J., Norby, R.J., Wullschlegel, S.D., 1993. Responses of loblolly pine seedlings to elevated CO<sub>2</sub> and fluctuating water supply. *Tree Physiol.* 13, 283–296.
- Van der Velde, M., Tubiello, F.N., Vrieling, A., Bouraoui, F., 2012. Impacts of extreme weather on wheat and maize in France: evaluating regional crop simulations against observed data. *Clim. Change* 113, 751–765.
- Walker, R.F., Geisinger, D.R., Johnson, D.W., Ball, J.T., 1997. Elevated atmospheric CO<sub>2</sub> and soil N fertility effects on growth, mycorrhizal colonization, and xylem water potential of juvenile ponderosa pine in a field soil. *Plant Soil* 195, 25–36.
- Weiss, I., Mizrahi, Y., Raveh, E., 2009. Synergistic effects of elevated CO<sub>2</sub> and fertilization on net CO<sub>2</sub> uptake and growth of the CAM plant *Hylocereus undatus*. *J. Am. Soc. Hortic. Sci.* 134, 364–371.
- Weiss, I., Mizrahi, Y., Raveh, E., 2010. Effect of elevated CO<sub>2</sub> on vegetative and reproductive growth characteristics of the CAM plants *Hylocereus undatus* and *Selenicereus megalanthus*. *Sci. Hortic.* 123, 531–536.
- West, A.G., Hultine, K.R., Sperry, J.S., Bush, S.E., Ehleringer, J.R., 2008. Transpiration and hydraulic strategies in a piñon–juniper woodland. *Ecol. Appl.* 18, 911–927.
- Widodo, W., Vu, J.C., Boote, K.J., Baker, J.T., Allen, L.H., 2003. Elevated growth CO<sub>2</sub> delays drought stress and accelerates recovery of rice leaf photosynthesis. *Environ. Exp. Bot.* 49, 259–272.
- Wiley, E., Helliker, B., 2012. A re-evaluation of carbon storage in trees lends greater support for carbon limitation to growth. *New Phytol.* 195, 285–289.
- Wong, S.C., 1990. Elevated atmospheric partial pressure of CO<sub>2</sub> and plant growth: II. Non-structural carbohydrate content in cotton plants and its effect on growth parameters. *Photosynth. Res.* 23, 171–180.
- Zinta, G., AbdElgawad, H., Domagalska, M.A., Vergauwen, L., Knapen, D., Nijs, I., Asard, H., 2014. Physiological, biochemical, and genome-wide transcriptional analysis reveals that elevated CO<sub>2</sub> mitigates the impact of combined heat wave and drought stress in *Arabidopsis thaliana* at multiple organizational levels. *Glob. Change Biol.* 20, 3670–3685.